

## MMSC1 - AN MMAC1 INTERACTING PROTEIN

### CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application is a divisional application of U.S. patent application Serial No. 09/233,086, filed January 19, 1999. The present application is related to U.S. provisional application 60/071,861, filed 20 January 1998, incorporated herein by reference, and claims priority thereto under 35 U.S.C. § 119(e).

### BACKGROUND OF THE INVENTION

[0002] The present invention is directed to the *MMSC1* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

[0003] The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

[0004] A number of genetic alterations are involved in the oncogenesis of glioblastoma multiforme, including inactivation of *p53*, *p16*, *RB*, amplification of the gene encoding epidermal growth factor receptor and several other molecular alterations (Louis & Gusella, 1995). However the most common genetic alteration is the deletion of large regions or an entire copy of chromosome 10 (Fults et al., 1990; Rahseed et al., 1992). Recently, the tumor suppressor gene *MMAC1* (Steck et al., 1997), also known as *PTEN* (Li et al., 1997) or *TEP1* (Li & Sun, 1997) was mapped to 10q23 and shown to be mutated in 17-24% of xenografted and primary glioblastomas, 14% of breast cancer samples and 25% of kidney carcinomas (Steck et al., 1997). The mutation frequency in established cell lines of these tumor types is somewhat higher. In addition to this predicted involvement in sporadic cancer, germ-line *MMAC1* mutations have been detected in two autosomal dominant disorders, Cowden disease (Nelen et al., 1997; Liaw et al., 1997), a syndrome that confers an elevated risk for tumors of breast, thyroid and skin, and Bannayan-Zonana syndrome (Marsh et al., 1997), a condition

characterized by macrocephaly, lipomas, intestinal hamartomatous polyps, vascular malformations and some skin disorders. Mutations of *MMAC1* in primary endometrial carcinomas (Kong et al., 1997) and in juvenile polyposis coli (Olschwang et al., 1998) have also been seen.

**[0005]** The predicted protein product of the *MMAC1* gene has several regions of homology with other proteins. The MMAC1 protein has an amino terminal domain with extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions, and with auxilin, a protein involved in synaptic vesicle transport. The MMAC1 protein also has a region with extensive homology to protein tyrosine phosphatases (Steck et al., 1997; Li et al., 1997). Mutations of *MMAC1* in tumors, its cytoplasmic localization (Li & Sun, 1997) and its intrinsic phosphatase activity (Li & Sun, 1997; Myers et al., 1997) suggested that its activity could be important in some aspect of tumor progression, possibly to counteract the oncogenic effect of a specific protein tyrosine kinase. In addition, *MMAC1* is rapidly down-regulated by TGF $\beta$  in cells sensitive to its cell growth and cell adhesion regulatory properties (Li & Sun, 1997).

**[0006]** Experiments on glioma cell growth have shown that MMAC1 is a protein phosphatase that exhibits functional and specific growth-suppressing activity. In such experiments, the introduction of HA-tagged MMAC1 into glioma cells containing endogenous mutant alleles caused growth suppression, but was without effect in cells containing HA-tagged MMAC1 (Furnari et al., 1997). The ectopic expression of *MMAC1* alleles, which carried mutations found in primary tumors and have been shown or are expected to inactivate its phosphatase activity, caused little growth suppression (Furnari et al., 1997). Although these activities of *MMAC1* are known, the mechanisms of tumor suppression by *MMAC1* and the interaction of the MMAC1 protein with other proteins are not well understood.

**[0007]** Many cytosolic signaling proteins and cytoskeletal proteins are composed of modular units of small protein-protein interactive domains that allow reversible and regulated assembly into larger protein complexes. These domains include the Src-homology SH2 and SH3 domains (Schlessinger, 1994; Pawson, 1994), pleckstrin-homology (PH) domains (Lemmon et al., 1996; Shaw, 1996), phosphotyrosine-binding (PTB) domains (Harrison,

1996; van der Greer & Pawson, 1995; Kavanaugh et al., 1995) and postsynaptic density protein, disc-large, zo-1 (PDZ) domains (Woods & Bryant, 1991; Dho et al., 1992; Woods & Bryant, 1993; Kennedy, 1995; Kornau et al., 1995). So far, PDZ domains have been found in more than 50 proteins (Tsunoda et al., 1997), and many proteins have multiple PDZ domains (Pawson & Scott, 1997). For a review of PDZ domains, as well as the other protein-protein interactive domains, see Pawson & Scott (1997).

**[0008]** A distinguishing feature of PDZ domains is their recognition of short peptides at the carboxyl terminal end of proteins. For example, one family of PDZ domains selected peptides with the consensus motif Glu-(Ser/Thr)-Xaa-(Val/Ile) (SEQ ID NO:1) at the carboxy terminus, whereas a second family of PDZ domains selected peptides with hydrophobic or aromatic side chains at the carboxy terminal three residues (Songyang et al., 1997). The presence of multiple PDZ domains in proteins may have at least two important consequences. An individual PDZ-containing protein could bind several subunits of a particular channel thereby inducing channel aggregations. Furthermore, the individual domains of a protein can have distinct binding specificities thereby inducing the formation of clusters that contain heterogeneous groups of proteins.

**[0009]** One example of this latter consequence of multiple PDZ domains is the InaD protein which contains five PDZ domains and acts as a scaffolding protein to organize the light-activated signaling events in *Drosophila* (Shieh & Zhu, 1996; Tsunoda et al., 1997). InaD associates through distinct PDZ domains with a calcium channel (TRP), phospholipase C- $\beta$  (the target of rhodopsin-activated heterotrimeric guanine nucleotide-binding protein (Gq $\alpha$ )) and protein kinase C.

**[00010]** Two further properties of PDZ domains or proteins which contain them may expand their potential activities. First, some PDZ domains may bind internal peptide sequences and, indeed, have a propensity to undergo homotypic or heterotypic interactions with other PDZ domains (Brenman et al., 1996). Second, proteins with PDZ domains frequently contain other interaction modules, including SH3 and LIM domains, and catalytic elements such as a tyrosine phosphatase or nitric oxide synthase domains. PDZ domains may therefore both coordinate the localization and clustering of receptors and channels, and provide a bridge to the cytoskeleton or intracellular signaling pathways.

[00011] It is desired to determine the mechanisms of tumor suppression for *MMAC1* and to identify proteins which interact with the MMAC1 protein. Such proteins can be used to assay for mutated MMAC1 proteins and/or screen potential drugs for suppressing tumor growth and/or identify additional proteins which interact with MMAC1.

#### SUMMARY OF THE INVENTION

[00012] The present invention is directed to the *MMSC1* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

[00013] Using yeast two-hybrid screening, it has been found MMAC1 binds to a protein herein named MMSC1. The nucleotide sequence is set forth as SEQ ID NO:2, and the amino acid sequence is set forth as SEQ ID NO:3. It has been found MMSC1 has 11 PDZ domains and that one or more of these domains interacts specifically with the three carboxyl terminal amino acids of MMAC1. Specifically, it has been found that PDZ domain number 7 interacts with MMAC1. Since MMSC1 contains 11 PDZ domains and interacts with MMAC1, a known tumor suppressor having a region of homology with protein tyrosine phosphatases, MMSC1 acts as a scaffolding protein in a common biochemical pathway with MMAC1. These characteristics indicate that the interaction between MMAC1 and MMSC1 is required for the tumor suppressor activity of *MMAC1*.

#### BRIEF DESCRIPTION OF THE FIGURES

[00014] Figure 1 shows a diagram of *MMSC1* indicating the position of the 11 PDZ domains and the overlap of the two mouse cDNA clones.

[00015] Figure 2 shows an alignment of the first 300 nucleotides of human *MMSC1* (H.s.\_MMSC1) with its translation product (H.s.\_MMSC1.pep) and the corresponding sequence from the mouse ortholog (M.m.\_MMSC1; SEQ ID NO:4), as determined from an analysis of the sequence from the above noted clones, with its translation product (M.m.\_MMSC1.pep; SEQ ID NO:5).

### SUMMARY OF SEQUENCE LISTING

[00016] SEQ ID NO:1 is a consensus motif to which one family of PDZ domains interacts. SEQ ID NO:2 is the nucleotide sequence for the *MMSC1* gene. SEQ ID NO:3 is the amino acid sequence for the MMSC1 protein. SEQ ID NO:4 is the nucleotide sequence for the 5' end of a fragment of the mouse homolog. SEQ ID NO:5 is the amino acid sequence for the N-terminus fragment of a mouse homolog. SEQ ID NO:6 is the 15 C-terminal amino acids of MMAC1. SEQ ID NO:7 is the SH3 binding peptide. SEQ ID NO:8 is the AF6 binding peptide. SEQ ID NO:9 is the MMAC1 binding peptide. SEQ ID NOs:10-65 are primers for PCR amplification of the *MMSC1* gene.

### DETAILED DESCRIPTION OF THE INVENTION

[00017] The present invention is directed to the *MMSC1* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

[00018] Using yeast two-hybrid screening, it has been found MMAC1 binds to a protein herein named MMSC1. The nucleotide sequence is set forth as SEQ ID NO:2, and the amino acid sequence is set forth as SEQ ID NO:3. It has been found MMSC1 has 11 PDZ domains and that one or more of these domains interacts specifically with the three carboxyl terminal amino acids of MMAC1. Specifically, it has been found that PDZ domain number 7 interacts with MMAC1. Since MMSC1 contains 11 PDZ domains and interacts with MMAC1, a known tumor suppressor having a region of homology with protein tyrosine phosphatases, MMSC1 acts as a scaffolding protein in a common biochemical pathway with MMAC1. These characteristics indicate that the interaction between MMAC1 and MMSC1 is required for the tumor suppressor activity of *MMAC1*.

[00019] The evidence presented herein shows that the function of MMSC1 is to make a scaffold that binds to MMAC1, the phosphatase substrate(s), and the (probably oncogene) tyrosine kinase(s). Thus, a valuable drug will be one that can prevent binding of either the substrate(s) or the tyrosine kinases(s) to MMSC1.

**[00020]** The yeast two-hybrid screening assay described herein identified two clones encoding *bona fide* MMAC1 interacting proteins. The clones were identified *pzdk5* and *pdzk21*. A search of GenBank with the sequences of *pzdk5* and *pdzk21* revealed that they could be assembled with a partial cDNA sequence AJ001306, to generate the complete coding sequence of a gene named *MMSC1* set forth in SEQ ID NO:2. dBEST sequences from two mouse cDNA clones (GenBank accession numbers AA030135 and W50755; IMAGE clone numbers 457904 and 356188) suggested that they might contain the start and stop codons, respectively, of the mouse ortholog of *MMSC1*. Sequencing of these clones revealed that this was indeed the case and confirmed the assignment of the translation start and stop codons in *MMSC1*.

**[00021]** As previously noted, SEQ ID NO:2 sets forth the nucleotide sequence for *MMSC1*. However, it has been found that the mRNA for *MMSC1* is subject to alternate splicing. On the basis of the sequence for *MMSC1*, genomic clones have been isolated and are being analyzed to determine splice junctions and alternate splicing for the mRNA. In addition, the PDZ domains of *MMSC1* are analyzed in the yeast two-hybrid assay to identify other proteins which interact with *MMSC1* and consequently are involved in the MMAC1 tumor suppressor pathway.

**[00022]** Since *MMSC1* is an MMAC1 interacting protein that is involved in tumor suppression activity in the *MMAC1* pathway, mutations in the *MMSC1* gene which affect the interaction of *MMSC1* with MMAC1 or affect the interaction of other proteins with MMAC1 as a result of the scaffolding effect of *MMSC1* will interfere with the MMAC1 tumor suppressor pathway and lead to tumorigenesis. Thus, an additional aspect of the present invention is the screening of *MMSC1* for such mutations using conventional techniques. Such methods may further comprise the step of amplifying a portion of the *MMSC1* gene, and may further include a step of providing a set of polynucleotides which are primers for amplification of said portion of the *MMSC1* gene. The method is useful for identifying mutations for use in either diagnosis of cancer or prognosis of cancer. Since such variants can now be detected earlier, i.e., before symptoms appear, and more definitively, better treatment options will be available in those individuals identified as having harmful mutations in *MMSC1*.

[00023] The present invention is directed to the determination that the MMSC1 binds to the C-terminal region of MMAC1 and is involved in a common pathway with MMAC1 which is a known tumor suppressor. Since many of the mutations in *MMAC1* are frameshift or nonsense mutations which consequently alter the C-terminus of MMAC1, MMSC1 can be used to assay for normal or mutated MMAC1 proteins using conventional techniques.

[00024] Finally, the present invention is directed to a method for screening drug candidates to identify drugs useful for treating or preventing cancer. Drug screening is performed by expressing mutant MMSC1 and assaying the effect of a drug candidate on the binding of MMSC1 with MMAC1. Similarly, one can test the effect of a drug candidate on the binding of wild-type MMSC1 with a mutant MMAC1. Such assays can be performed *in vitro* or *in vivo*, such as in oocytes, mammalian cells or transgenic animals. Other assays may test the ability of a drug, wherein the drug may be, e.g., a peptide, to replace the activity of MMSC1 such that the drug plus MMAC1 will work in concert similar to the normal wild-type interactions of MMSC1 and MMAC1. Again, similar assays may be performed to screen for drugs which replace a mutant MMAC1 and will bind to wild-type MMSC1 to replace the MMAC1 function which is lacking as a result of a mutated MMAC1.

[00025] According to the diagnostic and prognostic method of the present invention, alteration of the wild-type *MMSC1* gene is detected. In addition, the method can be performed by detecting the wild-type *MMSC1* gene and confirming the lack of a cause of cancer as a result of this locus. "Alteration of a wild-type gene" encompasses all forms of mutations including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Somatic mutations are those which occur only in certain tissues and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the *MMSC1* gene product, or to a decrease in mRNA stability or translation efficiency.

[00026] Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis, hybridization using nucleic acid modified with gold nanoparticles and PCR-SSCP, as discussed in detail further below. Also useful is the recently developed technique of DNA microchip technology.

[00027] The presence of cancer due to a germline mutation at this locus may be ascertained by testing any tissue of a human for mutations of the *MMSC1* gene. For example, a person who has inherited a germline *MMSC1* mutation, especially one which alters the interaction of *MMSC1* with *MMAC1*, would be prone to develop cancer. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *MMSC1* gene. Alteration of a wild-type *MMSC1* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

[00028] There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect



these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

**[00029]** A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of cancer cases. Southern blots displaying hybridizing fragments differing in length from control DNA when probed with sequences near or including the *MMSC1* locus indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

**[00030]** Detection of point mutations may be accomplished amplification, e.g., PCR, from genomic or cDNA and sequencing the amplified nucleic acid or by molecular cloning of the *MMSC1* allele and sequencing the allele using techniques well known in the art.

**[00031]** There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Rano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *MMSC1* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions

and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

**[00032]** In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

**[00033]** Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *MMSC1* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or

DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

**[00034]** In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *MMSC1* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

**[00035]** DNA sequences of the *MMSC1* gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *MMSC1* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe.

**[00036]** The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously

increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene BRCA1 (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Nature Genetics, 1996). Also see Fodor (1997).

[00037] The most definitive test for mutations in a candidate locus is to directly compare genomic *MMSC1* sequences from patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

[00038] Mutations from patients falling outside the coding region of *MMSC1* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

[00039] Alteration of *MMSC1* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type *MMSC1* protein. For example, monoclonal antibodies immunoreactive with *MMSC1* can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered *MMSC1* protein can be used to detect alteration of the wild-type *MMSC1* gene. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect *MMSC1* biochemical function. Finding a mutant *MMSC1* gene product indicates alteration of a wild-type *MMSC1* gene. One such binding assay is the binding of *MMSC1* with wild-type *MMAC1*. Conversely, wild-type *MMSC1* or the PDZ domain interacting with *MMAC1* can be used in a protein binding assay or biochemical

function assay to detect normal or mutant MMAC1 proteins, where the mutant proteins are proteins lacking a wild-type C-terminus.

[00040] A mutant *MMSC1* gene or gene product or a mutant MMAC1 can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for cancer resulting from a mutation in the *MMSC1* gene.

[00041] The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular *MMSC1* allele using PCR. The pairs of single-stranded DNA primers for *MMSC1* can be annealed to sequences within or surrounding the *MMSC1* gene in order to prime amplifying DNA synthesis of the gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular *MMSC1* mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

[00042] In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Alternatively, primers can also be prepared with 5' phosphoryl groups which will allow for blunt end cloning of amplified sequences. Thus, all nucleotides of the primers are derived from *MMSC1* sequence or sequences adjacent to *MMSC1*, except for the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of *MMSC1*, design of particular primers is well within the skill of the art.

[00043] The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The

probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the *MMSC1* gene or mRNA using other techniques.

**[00044]** Mutations which interfere with the function of the *MMSC1* gene product are involved in the pathogenesis of cancer. Thus, the presence of an altered (or a mutant) *MMSC1* gene which produces a protein having a loss of function, or altered function, directly increases the risk of cancer. In order to detect a *MMSC1* gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the allele being analyzed and the sequence of the wild-type allele. Mutant *MMSC1* alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alternatively, mutant alleles can be initially identified by identifying mutant (altered) proteins, using conventional techniques. The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the protein, are then used for the diagnostic and prognostic methods of the present invention.

**[00045]** The identification of the association between the *MMSC1* gene mutations and cancer permits the early presymptomatic screening of individuals to identify those at risk for developing cancer. To identify such individuals, *MMSC1* alleles are screened for mutations either directly or after cloning the alleles. The alleles are tested for the presence of nucleic acid sequence differences from the normal allele using any suitable technique, including but not limited to, one of the following methods: fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCP), linkage analysis, RNase protection assay, allele specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP analysis. Also useful is the recently developed technique of DNA microchip technology. For example, either (1) the nucleotide sequence of both the cloned alleles and normal *MMSC1* gene or appropriate fragment (coding sequence or genomic sequence) are determined and then compared, or (2) the RNA transcripts of the *MMSC1* gene or gene fragment are hybridized to single stranded whole genomic DNA from an individual to be tested, and the resulting heteroduplex is treated with Ribonuclease A (RNase A) and run on a denaturing gel to detect the location of any mismatches. Two of these methods can be carried out according to the following procedures.

[00046] The alleles of the *MMSC1* gene in an individual to be tested are cloned using conventional techniques. For example, a blood sample is obtained from the individual. The genomic DNA isolated from the cells in this sample is partially digested to an average fragment size of approximately 20 kb. Fragments in the range from 18-21 kb are isolated. The resulting fragments are ligated into an appropriate vector. The sequences of the clones are then determined and compared to the normal *MMSC1* gene.

[00047] Alternatively, polymerase chain reactions (PCRs) are performed with primer pairs for the 5' region or the exons of the *MMSC1* gene. PCRs can also be performed with primer pairs based on any sequence of the normal *MMSC1* gene. For example, primer pairs for one of the introns can be prepared and utilized. Finally, RT-PCR can also be performed on the mRNA. The amplified products are then analyzed by single stranded conformation polymorphisms (SSCP) using conventional techniques to identify any differences and these are then sequenced and compared to the normal gene sequence.

[00048] Individuals can be quickly screened for common *MMSC1* gene variants by amplifying the individual's DNA using suitable primer pairs and analyzing the amplified product, e.g., by dot-blot hybridization using allele-specific oligonucleotide probes.

[00049] The second method employs RNase A to assist in the detection of differences between the normal *MMSC1* gene and defective genes. This comparison is performed in steps using small (~500 bp) restriction fragments of the *MMSC1* gene as the probe. First, the *MMSC1* gene is digested with a restriction enzyme(s) that cuts the gene sequence into fragments of approximately 500 bp. These fragments are separated on an electrophoresis gel, purified from the gel and cloned individually, in both orientations, into an SP6 vector (e.g., pSP64 or pSP65). The SP6-based plasmids containing inserts of the *MMSC1* gene fragments are transcribed *in vitro* using the SP6 transcription system, well known in the art, in the presence of [ $\alpha$ -<sup>32</sup>P]GTP, generating radiolabeled RNA transcripts of both strands of the gene.

[00050] Individually, these RNA transcripts are used to form heteroduplexes with the allelic DNA using conventional techniques. Mismatches that occur in the RNA:DNA heteroduplex, owing to sequence differences between the *MMSC1* fragment and the *MMSC1* allele subclone from the individual, result in cleavage in the RNA strand when treated with RNase A. Such mismatches can be the result of point mutations or small deletions in the

individual's allele. Cleavage of the RNA strand yields two or more small RNA fragments, which run faster on the denaturing gel than the RNA probe itself.

[00051] Any differences which are found, will identify an individual as having a molecular variant of the *MMSC1* gene and the consequent presence of cancer. These variants can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function.

[00052] Genetic testing will enable practitioners to identify individuals at risk for cancer at, or even before, birth. Finally, this invention changes our understanding of the cause and treatment of cancer.

#### Definitions

[00053] The present invention employs the following definitions.

[00054] "**Amplification of Polynucleotides**" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. Also useful are strand displacement amplification (SDA), thermophilic SDA, and nucleic acid sequence based amplification (3SR or NASBA). These methods are well known and widely practiced in the art. See, e.g., U.S. Patents 4,683,195 and 4,683,202 and Innis *et al.*, 1990 (for PCR); Wu *et al.*, 1989a (for LCR); U.S. Patents 5,270,184 and 5,455,166 and Walker *et al.*, 1992 (for SDA); Spargo *et al.*, 1996 (for thermophilic SDA) and U.S. Patent 5,409,818, Fahy *et al.*, 1991 and Compton, 1991 for 3SR and NASBA. Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from the *MMSC1* region are preferably complementary to, and hybridize specifically to sequences in the *MMSC1* region or in



regions that flank a target region therein. *MMSC1* sequences generated by amplification may be sequenced directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf, 1986.

[00055]        "**Analyte polynucleotide**" and "**analyte strand**" refer to a single- or double-stranded polynucleotide which is suspected of containing a target sequence, and which may be present in a variety of types of samples, including biological samples.

[00056]        "**Antibodies.**" The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of specifically binding to the *MMSC1* polypeptide and fragments thereof or to polynucleotide sequences from the *MMSC1* region. The term "**antibody**" is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the *MMSC1* polypeptide or fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested for specific immunoreactivity with *MMSC1* polypeptide or fragments thereof. See, Harlow and Lane, 1988. These antibodies will be useful in assays as well as pharmaceuticals.

[00057]        Once a sufficient quantity of desired polypeptide has been obtained, it may be used for various purposes. A typical use is the production of antibodies specific for binding. These antibodies may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal and by other parameters well known to immunologists. Typical sites for injection are in footpads, intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

[00058] An immunological response is usually assayed with an immunoassay. Normally, such immunoassays involve some purification of a source of antigen, for example, that produced by the same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art. See, e.g., Harlow and Lane, 1988, or Goding, 1986.

[00059] Monoclonal antibodies with affinities of  $10^{-8}$  M<sup>-1</sup> or preferably  $10^{-9}$  to  $10^{-10}$  M<sup>-1</sup> or stronger will typically be made by standard procedures as described, e.g., in Harlow and Lane, 1988 or Goding, 1986. Briefly, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

[00060] Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse *et al.*, 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567).

[00061] **"Binding partner"** refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen and an antigen-specific antibody or an enzyme and its inhibitor. In general, the specific binding partners must bind with sufficient affinity to immobilize the analyte copy/complementary strand duplex (in the case of polynucleotide hybridization) under the isolation conditions. Specific binding partners are

known in the art and include, for example, biotin and avidin or streptavidin, IgG and protein A, the numerous, known receptor-ligand couples, and complementary polynucleotide strands. In the case of complementary polynucleotide binding partners, the partners are normally at least about 15 bases in length, and may be at least 40 bases in length. It is well recognized by those of skill in the art that lengths shorter than 15 (e.g., 8 bases), between 15 and 40, and greater than 40 bases may also be used. The polynucleotides may be composed of DNA, RNA, or synthetic nucleotide analogs. In addition, as disclosed herein, MMAC1 and PDZ binding peptides, as well as several other proteins, bind to or interact with MMSC1. Each of these proteins are also considered binding partners herein.

**[00062]** A **"biological sample"** refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide from an individual including, but not limited to, e.g., plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

**[00063]** **"Encode"**. A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

**[00064]** **"Isolated" or "substantially pure"**. An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

**[00065]** **"MMSC1 Allele"** refers to normal alleles of the *MMSC1* locus that interact with MMAC1 as well as alleles of *MMSC1* carrying variations that affect the interaction with MMAC1 and that cause cancer.

[00066] "*MMSC1* Locus", "*MMSC1* Gene", "*MMSC1* Nucleic Acids" or "*MMSC1* Polynucleotide" each refer to polynucleotides, all of which are in the *MMSC1* region, that are likely to be expressed in normal tissue, certain alleles of which adversely affect the interaction with MMAC1 and result in cancer. The *MMSC1* locus is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The *MMSC1* locus is intended to include all allelic variations of the DNA sequence.

[00067] These terms, when applied to a nucleic acid, refer to a nucleic acid which encodes a human MMSC1 polypeptide, fragment, homolog or variant, including, e.g., protein fusions or deletions. The nucleic acids of the present invention will possess a sequence which is either derived from, or substantially similar to a natural MMSC1-encoding gene or one having substantial homology with a natural MMSC1-encoding gene or a portion thereof.

[00068] The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

[00069] The present invention provides recombinant nucleic acids comprising all or part of the *MMSC1* region. The recombinant construct may be capable of replicating autonomously in a host cell. Alternatively, the recombinant construct may become integrated into the chromosomal DNA of the host cell. Such a recombinant polynucleotide comprises

a polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; 2) is linked to a polynucleotide other than that to which it is linked in nature; or 3) does not occur in nature.

**[00070]** Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this invention. Although the wild-type sequence may be employed, it will often be altered, e.g., by deletion, substitution or insertion. cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present invention, or such nucleic acids may be provided by amplification of sequences resident in genomic DNA or other natural sources, e.g., by PCR. The choice of cDNA libraries normally corresponds to a tissue source which is abundant in mRNA for the desired proteins. Phage libraries are normally preferred, but other types of libraries may be used. Clones of a library are spread onto plates, transferred to a substrate for screening, denatured and probed for the presence of desired sequences.

**[00071]** The DNA sequences used in this invention will usually comprise at least about five codons (15 nucleotides), more usually at least about 7-15 codons, and most preferably, at least about 35 codons. One or more introns may also be present. This number of nucleotides is usually about the minimal length required for a successful probe that would hybridize specifically with a MMSC1-encoding sequence. In this context, oligomers of as low as 8 nucleotides, more generally 8-17 nucleotides, can be used for probes, especially in connection with chip technology.

**[00072]** Techniques for nucleic acid manipulation are described generally, for example, in Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992. Reagents useful in applying such techniques, such as restriction enzymes and the like, are widely known in the art and commercially available from such vendors as New England BioLabs, Boehringer Mannheim, Amersham, Promega, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid sequences used to produce fusion proteins of the present invention may be derived from natural or synthetic sequences. Many natural gene sequences are obtainable from various cDNA or from genomic libraries using appropriate probes. See, GenBank, National Institutes of Health.

[00073] As used herein, a **"portion"** of the *MMSC1* locus or region or allele is defined as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides. This definition includes all sizes in the range of 8-40 nucleotides as well as greater than 40 nucleotides.

[00074] **"MMSC1 protein"** or **"MMSC1 polypeptide"** refers to a protein or polypeptide encoded by the *MMSC1* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides, oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native MMSC1 sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to MMSC1-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the MMSC1 protein(s).

[00075] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues.

[00076] **"Operably linked"** refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

[00077] **"Probes"**. Polynucleotide polymorphisms associated with *MMSC1* alleles which predispose to cancer are detected by hybridization with a polynucleotide probe which forms a stable hybrid with that of the target sequence, under highly stringent to moderately

stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, high stringency conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected with the result that the probe will not be completely complementary. Conditions are chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. (It should be noted that throughout this disclosure, if it is simply stated that "stringent" conditions are used that is meant to be read as "high stringency" conditions are used.) Since such indications identify neutral DNA polymorphisms as well as mutations, these indications need further analysis to demonstrate detection of a *MMSC1* susceptibility allele.

**[00078]** Probes for *MMSC1* alleles may be derived from the sequences of the *MMSC1* region or its cDNA. The probes may be of any suitable length, which span all or a portion of the *MMSC1* region, and which allow specific hybridization to the region. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even highly stringent conditions. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be employed which hybridizes to the target sequence with the requisite specificity.

**[00079]** The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate other polynucleotide sequences, having sequence similarity by standard methods. For techniques for preparing and labeling probes see, e.g., Sambrook et al., 1989 or Ausubel et al., 1992. Other similar polynucleotides may be selected by using homologous polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthesized or selected by use of the redundancy in the genetic code. Various codon substitutions may be introduced, e.g., by silent changes (thereby producing various restriction sites) or to optimize expression for a particular system. Mutations may be introduced to modify the properties of the polypeptide, perhaps to change the polypeptide degradation or turnover rate.

**[00080]** Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double-

stranded polynucleotides, or be chemically synthesized. Probes may also be labeled by nick translation, Klenow fill-in reaction, or other methods known in the art.

**[00081]** Portions of the polynucleotide sequence having at least about eight nucleotides, usually at least about 15 nucleotides, and fewer than about 9 kb, usually fewer than about 1.0 kb, from a polynucleotide sequence encoding *MMSC1* are preferred as probes. This definition therefore includes probes of sizes 8 nucleotides through 9000 nucleotides. The probes may also be used to determine whether mRNA encoding *MMSC1* is present in a cell or tissue.

**[00082]** **"Protein modifications or fragments"** are provided by the present invention for MMSC1 polypeptides or fragments thereof which are substantially homologous to primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as  $^{32}\text{P}$ , ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods of labeling polypeptides are well known in the art. See Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992.

**[00083]** Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. Significant biological activities include ligand-binding, immunological activity and other biological activities characteristic of MMSC1 polypeptides. Immunological activities include both immunogenic function in a target immune system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an epitope of the MMSC1 protein. As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation which is unique to the epitope.



Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of such amino acids are known in the art.

[00084] For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highly antigenic proteins. Alternatively, such polypeptides will serve as highly efficient competitors for specific binding. Production of antibodies specific for MMSC1 polypeptides or fragments thereof is described below.

[00085] The present invention also provides for fusion polypeptides, comprising MMSC1 polypeptides and fragments. Homologous polypeptides may be fusions between two or more MMSC1 polypeptide sequences or between the sequences of MMSC1 and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between different new fusion polypeptides or fragments. Such homologous or heterologous fusion polypeptides may display, for example, altered strength or specificity of binding. Fusion partners include immunoglobulins, bacterial  $\beta$ -galactosidase, trpE, protein A,  $\beta$ -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor. See Godowski *et al.*, 1988.

[00086] Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemically synthesized. Techniques for the synthesis of polypeptides are described, for example, in Merrifield, 1963.

[00087] "**Protein purification**" refers to various methods for the isolation of the MMSC1 polypeptides from other biological material, such as from cells transformed with recombinant nucleic acids encoding MMSC1, and are well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography employing, e.g., the antibodies provided by the present invention. Various methods of protein purification are well known in the art, and include those described in Deutscher, 1990 and Scopes, 1982.

[00088] The terms "**isolated**", "**substantially pure**", and "**substantially homogeneous**" are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide

sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

**[00089]** A MMSC1 protein is substantially free of naturally associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

**[00090]** A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypeptide," as used herein, even if expressed in a homologous cell type. Synthetically made forms or molecules expressed by heterologous cells are inherently isolated molecules.

**[00091]** **"Recombinant nucleic acid"** is a nucleic acid which is not naturally occurring, or which is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

**[00092]** **"Regulatory sequences"** refers to those sequences normally within 100 kb of the coding region of a locus, but they may also be more distant from the coding region, which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability or the like of the messenger RNA).

[00093] **"Substantial homology or similarity"**. A nucleic acid or fragment thereof is "substantially homologous" ("or substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases.

[00094] Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa, 1984. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

[00095] Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. See, e.g., Wetmur and Davidson, 1968.

[00096] Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

[00097] The terms "**substantial homology**" or "**substantial identity**", when referring to polypeptides, indicate that the polypeptide or protein in question exhibits at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity, and preferably at least about 95% identity.

[00098] "**Substantially similar function**" refers to the function of a modified nucleic acid or a modified protein, with reference to the wild-type *MMSC1* nucleic acid or wild-type *MMSC1* polypeptide. The modified polypeptide will be substantially homologous to the wild-type *MMSC1* polypeptide and will have substantially the same function. The modified polypeptide may have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the modified polypeptide may have other useful properties, such as a longer half-life. The similarity of function (activity) of the modified polypeptide may be substantially the same as the activity of the wild-type *MMSC1* polypeptide. Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type *MMSC1* polypeptide. The modified polypeptide is synthesized using conventional techniques, or is encoded by a modified nucleic acid and produced using conventional techniques. The modified nucleic acid is prepared by conventional techniques. A nucleic acid with a function substantially similar to the wild-type *MMSC1* gene function produces the modified protein described above.

[00099] Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measure of homology assigned to various substitutions, deletions and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

[000100] A polypeptide "**fragment**," "**portion**" or "**segment**" is a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more contiguous amino acids.

[000101] The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

[000102] "**Target region**" refers to a region of the nucleic acid which is amplified and/or detected. The term "**target sequence**" refers to a sequence with which a probe or primer will form a stable hybrid under desired conditions.

[000103] The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, and immunology. See, e.g., Maniatis *et al.*, 1982; Sambrook *et al.*, 1989; Ausubel *et al.*, 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991. A general discussion of techniques and materials for human gene mapping, including mapping of human chromosome 1, is provided, e.g., in White and Lalouel, 1988.

Preparation of recombinant or chemically synthesized  
nucleic acids; vectors, transformation, host cells

[000104] Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian, plant, insect or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992.

**[000105]** The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Carruthers, 1981 or the triester method according to Matteucci and Caruthers, 1981, and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

**[000106]** Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al., 1989 or Ausubel et al., 1992.

**[000107]** An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *MMSC1* gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al., 1989 or Ausubel et al., 1992; see also, e.g., Metzger et al., 1988. Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression

are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983).

**[000108]** While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

**[000109]** Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

**[000110]** The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al., 1989 and Ausubel et al., 1992. The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which

have been introduced nucleic acids described above are meant to also include the progeny of such cells.

[000111] Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the *MMSCI* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

[000112] Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.), 1979. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines. An example of a commonly used insect cell line is SF9. However, it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features.

[000113] Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

[000114] Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of MMSC1 polypeptide.

[000115] The probes and primers based on the *MMSCI* gene sequence disclosed herein are used to identify homologous *MMSCI* gene sequences and proteins in other species. These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.



Methods of Use: Drug Screening

**[000116]** This invention is particularly useful for screening compounds by using the MMSC1 polypeptide or binding fragment thereof in any of a variety of drug screening techniques. Since MMSC1 acts as a scaffold that binds to MMAC1, the phosphatase substrate(s) and the (probably oncogene) tyrosine kinase(s), a valuable drug candidate will be a drug that can prevent binding of either the substrate(s) or the tyrosine kinase(s) to MMSC1.

**[000117]** The MMSC1 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eukaryotic or procaryotic host cells which are stably transformed with recombinant polynucleotides expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, for the formation of complexes between a MMSC1 polypeptide or fragment and the agent being tested, or examine the degree to which the formation of a complex between a MMSC1 polypeptide or fragment and a known ligand, e.g., MMAC1, is aided or interfered with by the agent being tested.

**[000118]** Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a MMSC1 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the MMSC1 polypeptide or fragment, or (ii) for the presence of a complex between the MMSC1 polypeptide or fragment and a ligand, by methods well known in the art. In such competitive binding assays the MMSC1 polypeptide or fragment is typically labeled. Free MMSC1 polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to MMSC1 or its interference with or promotion of MMSC1:ligand binding, respectively. One may also measure the amount of bound, rather than free, MMSC1. It is also possible to label the ligand rather than the MMSC1 and to measure the amount of ligand binding to MMSC1 in the presence and in the absence of the drug being tested.

**[000119]** Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the MMSC1 polypeptides and is described in

detail in Geysen, PCT published application WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with MMSC1 polypeptide and washed. Bound MMSC1 polypeptide is then detected by methods well known in the art.

**[000120]** Purified MMSC1 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used to capture antibodies to immobilize the MMSC1 polypeptide on the solid phase.

**[000121]** This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of specifically binding the MMSC1 polypeptide compete with a test compound for binding to the MMSC1 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants of the MMSC1 polypeptide.

**[000122]** The above screening methods are not limited to assays employing only MMSC1 but are also applicable to studying MMSC1-protein complexes, e.g., the complex which occurs between MMSC1 and MMAC1. The effect of drugs on the activity of this complex, especially when either the MMSC1 or the MMSC1 binding protein (e.g., MMAC1) contains a mutation, is analyzed.

**[000123]** In accordance with these methods, the following assays are examples of assays which can be used for screening for drug candidates.

**[000124]** A mutant MMSC1 (*per se* or as part of a fusion protein) is combined with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC1 binds. This combining is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant MMSC1 with the wild-type protein is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC1*. This assay is useful where the wild-type protein is a tumor suppressor, such as MMAC1.

**[000125]** A wild-type MMSC1 (*per se* or as part of a fusion protein) is combined with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC1 binds.

This combining is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type MMSC1 with the wild-type protein is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC1*. This assay is useful where the wild-type protein is a tumor suppressor, such as *MMAC1*.

**[000126]** A mutant MMSC1 (*per se* or as part of a fusion protein) is combined with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC1 binds. This combining is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant MMSC1 with the wild-type protein is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC1*. This assay is useful if the protein is an oncoprotein or a substrate of the oncoprotein.

**[000127]** A wild-type MMSC1 (*per se* or as part of a fusion protein) is combined with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC1 binds. This combining is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type MMSC1 with the wild-type protein is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC1* or a cancer resulting from a mutation in *MMAC1*. This assay is useful if the protein is an oncoprotein or a substrate of the oncoprotein.

**[000128]** A mutant protein, which as a wild-type protein binds to MMSC1 (*per se* or as part of a fusion protein) is combined with a wild-type MMSC1 (*per se* or as part of a fusion protein). This combining is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant protein with the wild-type MMSC1 is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in the gene encoding the protein.

**[000129]** The polypeptide of the invention may also be used for screening compounds developed as a result of combinatorial library technology. Combinatorial library technology provides an efficient way of testing a potential vast number of different substances for ability

to modulate activity of a polypeptide. Such libraries and their use are known in the art. The use of peptide libraries is preferred. See, for example, WO 97/02048.

**[000130]** Briefly, a method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances. A difference in activity between the treated and untreated polypeptides is indicative of a modulating effect of the relevant test substance or substances.

**[000131]** Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g., in a yeast two-hybrid system (e.g., Bartel et al., 1993). This system may be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide. Alternatively, the screen could be used to screen test substances for binding to a MMSC1 specific binding partner, such as MMAC1, or to find mimetics of the MMSC1 polypeptide.

**[000132]** Following identification of a substance which modulates or affects polypeptide activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

**[000133]** Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition comprising such a substance, a method comprising administration of such a composition to a patient, e.g., for treatment (which may include preventative treatment) of cancer, use of such a substance in the manufacture of a composition for administration, e.g., for treatment of cancer, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

**[000134]** A substance identified using as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide small molecules are often preferred for many in vivo pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

**[000135]** The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a lead compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

**[000136]** There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g., by substituting each residue in turn. Alanine scans of peptide are commonly used to refine such peptide motifs. These parts or residues constituting the active region of the compound are known as its pharmacophore.

**[000137]** Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

**[000138]** In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

**[000139]** A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups

grafted onto it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

#### Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

**[000140]** In order to detect the presence of a *MMSC1* allele predisposing an individual to cancer, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of *MMSC1*. In order to detect the presence of cancer or as a prognostic indicator, a biological sample is prepared and analyzed for the presence or absence of mutant alleles of *MMSC1*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

**[000141]** Initially, the screening method involves amplification of the relevant *MMSC1* sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

**[000142]** The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

**[000143]** When the probes are used to detect the presence of the target sequences the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence, e.g., denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

**[000144]** Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region for *MMSC1*. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis et al., 1982 and Sambrook et al., 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadruplexes, etc., may be desired to provide the means of detecting target sequences.

**[000145]** Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, gold nanoparticles and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that

amplify the signal from the labeled moiety. A number of these variations are reviewed in, e.g., Matthews and Kricka, 1988; Landegren et al., 1988; U.S. Patent 4,868,105; and in EPO Publication No. 225,807.

**[000146]** As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a  $10^3$ - $10^6$  increase in sensitivity. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes, see Jablonski et al., 1986.

**[000147]** Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding *MMSC1*. Allele specific probes are also contemplated within the scope of this example and exemplary allele specific probes include probes encompassing the predisposing mutations of this disclosure.

**[000148]** In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. For methods for labeling nucleic acid probes according to this embodiment see Martin et al., 1990. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. For methods for labeling nucleic acid probes and their use in biotin-avidin based assays see Rigby et al., 1977 and Nguyen et al., 1992.

**[000149]** It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of



detecting *MMSC1*. Thus, in one example to detect the presence of *MMSC1* in a cell sample, more than one probe complementary to the gene is employed and in particular the number of different probes is alternatively two, three, or five different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *MMSC1* gene sequence in a patient, more than one probe complementary to these genes is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *MMSC1*. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to cancer.

#### Methods of Use: Peptide Diagnosis and Diagnostic Kits

**[000150]** The presence of cancer can also be detected on the basis of the alteration of wild-type *MMSC1* polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of *MMSC1* peptides. Techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparations claimed in this invention. In a preferred embodiment of the invention, antibodies will immunoprecipitate *MMSC1* proteins from solution as well as react with these proteins on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect *MMSC1* proteins in paraffin or frozen tissue sections, using immunocytochemical techniques.

**[000151]** Preferred embodiments relating to methods for detecting *MMSC1* or its mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

**[000152]** Alternatively, alterations in the *MMSC1* sequence can be determined by detecting alterations in the interaction of *MMSC1* with *MMAC1* or the C-terminus of *MMAC1*. Wild-type *MMAC1* or its C-terminus can be bound to a solid phase and the

interaction with MMSC1 assayed by conventional techniques. Analogously, alterations in MMAC1 which affect its interaction with MMSC1 can be detected using wild-type MMSC1 or its PDZ domain which interacts with MMAC1 bound to a solid phase.

#### Methods of Use: Rational Drug Design

**[000153]** The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., MMSC1 polypeptide) by x-ray crystallography, by computer modeling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson *et al.*, 1990). In addition, peptides (e.g., MMSC1 polypeptide) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

**[000154]** It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

**[000155]** Thus, one may design drugs which have, e.g., improved MMSC1 polypeptide activity or stability or which act as inhibitors, agonists, antagonists, etc. of MMSC1 polypeptide activity. By virtue of the availability of cloned *MMSC1* sequence, sufficient

amounts of the MMSC1 polypeptide may be made available to perform such analytical studies as x-ray crystallography. In addition, the knowledge of the MMSC1 protein sequence provided herein will guide those employing computer modeling techniques in place of, or in addition to x-ray crystallography.

#### Methods of Use: Gene Therapy

[000156] According to the present invention, a method is also provided of supplying wild-type MMSC1 function to a cell which carries a mutant *MMSC1* allele. Supplying such a function should allow normal functioning of the recipient cells. The wild-type gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. More preferred is the situation where the wild-type gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous mutant gene present in the cell. Such recombination requires a double recombination event which results in the correction of the gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the practitioner.

[000157] As generally discussed above, the *MMSC1* gene or fragment, where applicable, may be employed in gene therapy methods in order to increase the amount of the expression products of such gene in cells. It may also be useful to increase the level of expression of the *MMSC1* gene even in those persons in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

[000158] Gene therapy would be carried out according to generally accepted methods, for example, as described by Friedman, 1991. Cells from a patient would be first analyzed by the diagnostic methods described above, to ascertain the production of MMSC1 polypeptide in the cells. A virus or plasmid vector (see further details below), containing a copy of the *MMSC1* gene linked to expression control elements and capable of replicating inside the cells, is prepared. Suitable vectors are known, such as disclosed in U.S. Patent

5,252,479 and PCT published application WO 93/07282. The vector is then injected into the patient. If the transfected gene is not permanently incorporated into the genome of each of the targeted cells, the treatment may have to be repeated periodically.

**[000159]** Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson et al., 1992; Stratford-Perricaudet et al., 1990), vaccinia virus (Moss, 1992), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakfield and Geller, 1987; Freese et al., 1990), and retroviruses of avian (Brandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992). Most human gene therapy protocols have been based on disabled murine retroviruses.

**[000160]** Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer et al., 1980); mechanical techniques, for example microinjection (Anderson et al., 1980; Gordon et al., 1980; Brinster et al., 1981; Constantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al., 1989; Stewart et al., 1992; Nabel et al., 1990; Lim et al., 1992); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990; Wu et al., 1991; Zenke et al., 1990; Wu et al., 1989b; Wolff et al., 1991; Wagner et al., 1990; Wagner et al., 1991; Cotten et al., 1990; Curiel et al., 1991a; Curiel et al., 1991b).

**[000161]** In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient

binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

**[000162]** Liposome/DNA complexes have been shown to be capable of mediating direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992).

**[000163]** Gene transfer techniques which target DNA directly to brain tissue is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

**[000164]** The therapy is as follows: patients who carry a *MMSC1* susceptibility allele are treated with a gene delivery vehicle such that some or all of their brain precursor cells receive at least one additional copy of a functional normal *MMSC1* allele, respectively. In this step, the treated individuals have reduced risk of cancer to the extent that the effect of the susceptible allele has been countered by the presence of the normal allele.

#### Methods of Use: Peptide Therapy

**[000165]** Peptides which have MMSC1 activity can be supplied to cells which carry a mutant or missing MMSC1 allele. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, MMSC1 polypeptide can be extracted from MMSC1-producing mammalian cells. In addition, the techniques of synthetic chemistry can be employed to synthesize MMSC1 protein. Any of such techniques can provide the preparation of the present invention which comprises the MMSC1 protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

**[000166]** Active MMSC1 molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Supply of molecules with MMSC1 activity should lead to inhibition of cancer. Other molecules with MMSC1 activity (for example, peptides, drugs or organic compounds) may also be used to effect such an inhibition. Modified polypeptides having substantially similar function are also used for peptide therapy.

#### Methods of Use: Transformed Hosts

**[000167]** Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant *MMSC1* alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous *MMSC1* gene of the animals may be disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989; Valancius and Smithies, 1991; Hasty *et al.*, 1991; Shinkai *et al.*, 1992; Mombaerts *et al.*, 1992; Philpott *et al.*, 1992; Snouwaert *et al.*, 1992; Donehower *et al.*, 1992). After test substances have been administered to the animals, the presence of cancer must be assessed. If the test substance prevents or suppresses the appearance of cancer, then the test substance is a candidate therapeutic agent for treatment of cancer. These animal models provide an extremely important testing vehicle for potential therapeutic products.

#### Methods of Use: Transgenic/Knockout Animals and Models

**[000168]** In one embodiment of the invention, transgenic animals are produced which contain a functional transgene encoding a functional MMSC1 polypeptide or variants thereof. Transgenic animals expressing MMSC1 transgenes, recombinant cell lines derived from such animals and transgenic embryos may be useful in methods for screening for and identifying agents that induce or repress function of MMSC1. Transgenic animals of the present invention also can be used as models for studying indications such as cancers.

**[000169]** In one embodiment of the invention, a MMSC1 transgene is introduced into a non-human host to produce a transgenic animal expressing a human or murine MMSC1 gene.

The transgenic animal is produced by the integration of the transgene into the genome in a manner that permits the expression of the transgene. Methods for producing transgenic animals are generally described by Wagner and Hoppe (U.S. Patent No. 4,873,191; which is incorporated herein by reference), Brinster *et al.* 1985; which is incorporated herein by reference in its entirety) and in "Manipulating the Mouse Embryo; A Laboratory Manual" 2nd edition (eds., Hogan, Beddington, Costantini and Long, Cold Spring Harbor Laboratory Press, 1994; which is incorporated herein by reference in its entirety).

**[000170]** It may be desirable to replace the endogenous MMSC1 by homologous recombination between the transgene and the endogenous gene; or the endogenous gene may be eliminated by deletion as in the preparation of "knock-out" animals. Typically, a MMSC1 gene flanked by genomic sequences is transferred by microinjection into a fertilized egg. The microinjected eggs are implanted into a host female, and the progeny are screened for the expression of the transgene. Transgenic animals may be produced from the fertilized eggs from a number of animals including, but not limited to reptiles, amphibians, birds, mammals, and fish. Within a particularly preferred embodiment, transgenic mice are generated which overexpress MMSC1 or express a mutant form of the polypeptide. Alternatively, the absence of a MMSC1 in "knock-out" mice permits the study of the effects that loss of MMSC1 protein has on a cell *in vivo*. Knock-out mice also provide a model for the development of MMSC1-related cancers.

**[000171]** Methods for producing knockout animals are generally described by Shastry (1995, 1998) and Osterrieder and Wolf (1998). The production of conditional knockout animals, in which the gene is active until knocked out at the desired time is generally described by Feil *et al.* (1996), Gagneten *et al.* (1997) and Lobe and Nagy (1998). Each of these references is incorporated herein by reference.

**[000172]** As noted above, transgenic animals and cell lines derived from such animals may find use in certain testing experiments. In this regard, transgenic animals and cell lines capable of expressing wild-type or mutant MMSC1 may be exposed to test substances. These test substances can be screened for the ability to enhance wild-type MMSC1 expression and or function or impair the expression or function of mutant MMSC1.

### Pharmaceutical Compositions and Routes of Administration

**[000173]** The MMSC1 polypeptides, antibodies, peptides and nucleic acids of the present invention can be formulated in pharmaceutical compositions, which are prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral, intrathecal, epineural or parenteral.

**[000174]** For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

**[000175]** For parenteral administration, the compound may dissolved in a pharmaceutical carrier and administered as either a solution of a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for



example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

**[000176]** The active agent is preferably administered in an therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or spealists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in *Remington's Parmaceutical Sciences*.

**[000177]** Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

**[000178]** Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and published PCT application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. designed for implantation in a patient. The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are more tissue specific to the target cells. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the active agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See for example, EP 425,731A and WO 90/07936.

## EXAMPLES

[000179] The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

## EXAMPLE 1

Identification of *MMSC1*

[000180] A yeast two-hybrid assay was performed using conventional techniques, such as described by Fields and Song (1989), Chevray and Nathans (1992), Bartel et al. (1993) and Lee et al. (1995). Sequence encoding the C-terminal 15 amino acids of MMAC1 (NEPFDEDQHTQITKV; SEQ ID NO:6) plus its stop codon was generated using an oligonucleotide synthesizer and was ligated to plasmid pGBT.C such that the coding sequence of *MMAC1* was in-frame with coding sequence for the Gal4p DNA-binding domain. This plasmid construct was introduced into the yeast reporter strain J692 along with a library of activation domain fusion plasmids prepared from human kidney cDNA (Clontech). Transformants were spread onto 20 - 150 mm plates of yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole (Gietz et al., 1995; Bartel and Fields, 1995). After one week incubation at 30°C, yeast colonies were assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay (Breen and Naysmyth, 1985). Colonies that both grew in the absence of histidine and were positive for production of beta-galactosidase were chosen for further characterization.

[000181] The activation domain plasmid was purified from positive colonies by the smash-and-grab technique. These plasmids were introduced into *E. coli* DH10B (Gibco BRL) by electroporation and purified from *E. coli* by the alkaline lysis method. To test for the specificity of the interaction, specific activation domain plasmids were cotransformed into strain J692 with plasmids encoding various DNA-binding domain fusion proteins, including fusions to C-terminal segments of MMAC1 and human lamin C. Transformants from these experiments were assayed for expression of the *HIS3* and *lacZ* reporter genes.

Positives that expressed reporter genes with *MMAC1* constructs and not with lamin C constructs encode bona fide MMAC1-interacting proteins. These proteins were identified and characterized by sequence analysis of the insert of the appropriate activation domain plasmid.

**[000182]** Two of the clones encoding bona fide MMAC1-interacting proteins were named pdzk5 and pdzk21. A search of GenBank with the sequences of pdzk5 and pdzk21 revealed that they could be assembled with a partial cDNA sequence, AJ001306, to generate the complete coding sequence of pdzk5. dbEST sequences from two mouse cDNA clones (GenBank accession numbers AA30135 and W50755; IMAGE clone numbers 457904 and 356188) suggested that they might contain the start and stop codons, respectively, of the mouse ortholog of pdzk5. Sequencing of these clones revealed that this was indeed the case and confirmed our assignment of the translational start and stop codons. The nucleotide sequence for *MMSC1* is set forth in SEQ ID NO:2 with the amino acid sequence of the encoded protein set forth in SEQ ID NO:3.

**[000183]** Figure 1 shows a diagram of *MMSC1* indicating the position of the 11 PDZ domains and the overlap of the two mouse cDNA clones. Figure 2 shows an alignment of the first 300 nucleotides of human *MMSC1* (H.s.\_MMSC1) with its translation product (H.s.\_MMSC1.pep) and the corresponding sequence from the mouse ortholog (M.m.\_MMSC1; SEQ ID NO:4), as determined from an analysis of the sequence from the above noted clones, with its translation product (M.m.\_MMSC1.pep; SEQ ID NO:5). Gaps have been introduced into the mouse sequence to optimize the alignment. The in-frame stop codon at nucleotide 93, shared by the mouse and human sequences, demonstrates that the start codon at nucleotide 115 has been correctly identified. The 11 PDZ domains correspond to the amino acids of *MMSC1* as shown in Table 1.

TABLE 1

Sequence Correspondence of 11 PDZ Domains

<u>Domain Number</u>	<u>Amino Acid Span</u>
1	133 - 219
2	247 - 326
3	364 - 451
4	558 - 637
5	685 - 771
6	1067 - 1158
7	1238 - 1320
8	1436 - 1518
9	1532 - 1613
10	1675 - 1760
11	1798 - 1882

**[000184]** The nucleotide sequence of *MMSC1* was compared with the sequence of GenBank accession number AJ001306. Other than sequencing errors, the following major differences were noted. First, the AJ001306 is missing at least one exon and possibly more (nucleotides 4492-4575 of SEQ ID NO:2). The absence of this exon knocks out PDZ domain number 8. Second, the AJ001306 sequence is either alternatively spliced or unspliced at nucleotide 4770 (relative to sEQ ID NO:2) which results in a stop codon at nt 4771 (relative to SEQ ID NO:2). This would knock out PDZ domains 9, 10 and 11.

## EXAMPLE 2

Identification of MMSC1-Interacting Proteins by Two-Hybrid Analysis

**[000185]** DNA fragments encoding all or portions of MMSC1 are ligated to a two-hybrid DNA-binding domain vector such as pGBT.C such that the coding sequence of *MMSC1* is in-frame with coding sequence for the Gal4p DNA-binding domain. These DNA fragments may encode specific PDZ domains of MMSC1 plus the 5 to 10 amino acids N- and C-terminal of each specific PDZ. A plasmid that encodes a DNA-binding domain fusion to a fragment of MMSC1 PDZ is introduced into the yeast reporter strain (such as J692) along with a library of cDNAs fused to an activation domain. Transformants are spread onto 20 - 150 mm plates of selective media, such as yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole. After one week incubation at

30° C, yeast colonies are assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay. Colonies that both grow in the absence of histidine and are positive for production of beta-galactosidase are chosen for further characterization.

**[000186]** The activation domain plasmid is purified from positive colonies by the smash-and-grab technique. These plasmids are introduced into *E. coli* (e.g., DH10B (Gibco BRL)) by electroporation and purified from *E. coli* by the alkaline lysis method. To test for the specificity of the interaction, specific activation domain plasmids are cotransformed into strain J692 with plasmids encoding various DNA-binding domain fusion proteins, including fusions to segments of MMSC1 and human lamin C. Transformants from these experiments are assayed for expression of the *HIS3* and *lacZ* reporter genes. Positives that express reporter genes with *MMSC1* constructs and not with lamin C constructs encode bona fide MMSC1-interacting proteins. These proteins are identified and characterized by sequence analysis of the insert of the appropriate activation domain plasmid.

### EXAMPLE 3

#### Characterization of the Binding Specificity of MMSC1 PDZ Domains by Two-Hybrid Analysis

**[000187]** DNA fragments encoding specific PDZ domains of MMSC1 plus the 5 to 10 amino acids N- and C-terminal of each specific PDZ domain are generated by PCR amplification. These fragments are ligated to a two-hybrid DNA-binding domain vector such as pGBT.C such that the coding sequence of *MMSC1* is in-frame with coding sequence for the Gal4p DNA-binding domain. An activation domain library is prepared that encodes an activation domain fused in-frame to random peptide sequences that end with a stop codon. An example of this type of library is the Clontech random peptide library. A plasmid that encodes a DNA-binding domain fusion to a specific MMSC1 PDZ domain is introduced into the yeast reporter strain (such as J692) along with a library of random peptides fused to an activation domain. Transformants are spread onto 20 - 150 mm plates of selective media, such as yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole. After one week incubation at 30° C, yeast colonies are assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay. Colonies that both

grow in the absence of histidine and are positive for production of beta-galactosidase are chosen for sequence analysis. The insert of the activation domain construct is characterized by sequence analysis. The sequence of the peptide that binds to the MMSC1 PDZ domain is obtained by conceptual translation of the nucleotide sequence. Peptide sequences from multiple isolates are aligned to determine a consensus binding motif. This motif can be used to identify cellular proteins that bind MMSC1 and to develop small molecules that inhibit binding to these specific PDZ domains.

#### EXAMPLE 4

##### *In vitro* Protein-Protein Interaction Assay

**[000188]** cDNAs encoding each of the MMSC1 PDZ domains (amino acid residues identified in Table 1), and any desired control proteins, were generated by PCR and subcloned as glutathione S-transferase (GST) fusions in pGEX vectors (Pharmacia). After sequencing to confirm expression construct integrity, the resulting clones were expressed in *E. coli* and the desired fusion proteins isolated with glutathione-agarose and recovered with glutathione elution. These fusion proteins or control proteins were then adsorbed to different wells of a 96-well ELISA plate and remaining sites blocked with BSA. Synthetic commercially synthesized peptides encoding the desired PDZ-binding domain (i.e., the 16 C-terminal amino acids of MMAC1, or the C-terminal peptide sequences of interacting proteins identified by the approach of Example 2, or the C-terminal peptide sequences identified by the approach of Example 3), or a control peptide, and biotinylated at the amino-terminus, were pre-bound to streptavidin-alkaline phosphatase in a 4:1 molar ratio. The biotinylated peptide streptavidin-alkaline phosphatase complexes were then blocked with free biotin. These pre-bound peptide streptavidin-alkaline phosphatase complexes were then incubated with the immobilized PDZ domains in wash buffer containing PBS, BSA and triton-X100. Unbound material was removed with repeated washes. Bound peptide/streptavidin-alkaline phosphatase complex was then quantitated by a colorimetric phosphatase assay read on a 96-well plate reader.

[000189] The following peptides were used in the initial study:

SH3 binding peptide                      biotin-SGSGILAPPVPPRNTR-COOH (SEQ ID NO:7)

AF6 PDZ binding peptide   biotin-SGDDGDDPFLQYEFYV-COOH (SEQ ID NO:8)

MMAC1.388-403                      biotin-ENEPFDEDQHTQITKV-COOH (SEQ ID NO:9).

[000190] The results of the peptide binding ELISA assay is set forth in Table 2.

TABLE 2

PDZ Binding Assay

<u>MMSc1 PDZ</u>	<u>Peptide</u>	<u>A405</u>
3	SH3	0.01
	AF6	2.24
	MMAC	0.01
5	SH3	0.00
	AF6	0.02
	MMAC	0.00
6	SH3	0.00
	AF6	1.46
	MMAC	0.23
7	SH3	0.01
	AF6	1.05
	MMAC	1.25
8	SH3	0.00
	AF6	0.29
	MMAC	0.30
9	SH3	0.02
	AF6	2.41
	MMAC	0.10

[000191] The GST-affinity pull down assay is a complementary *in vitro* method for investigating protein-protein interactions. PDZ domain-GST fusion proteins are incubated with synthetic biotinylated peptides in wash buffer (these peptides were described above). Streptavidin magnetic beads are then added to recover the biotinylated peptide, then unbound material removed by washing. The beads are then incubated with SDS/DTT loading buffer at 100° C and bound protein detected by SDS/PAGE and coomassie blue staining.

## EXAMPLE 5

Mutation screening of *MMSC1*

**[000192]** Nested PCR amplifications were performed on cDNA from tumor cell lines. Total cell line RNAs were reverse transcribed with Superscript II (Life Technologies) and random hexamers. Using the outer primer pair from each amplicon (i.e. PDZK5.1A and PDZK5.1P or PDZK5.2A and PDZK5.2P), approximately 10 ng of cDNA from each cell line was amplified for 26 cycles. Products were diluted 60 fold and then reamplified for 22-26 cycles using nested M13 tailed primers (i.e. PDZK5.1B and PDZK5.1Q or PDZK5.2B and PDZK5.2Q). Typical primary amplification cycling conditions were an initial denaturation at 95° for 60s, followed by 26 cycles of 96° (12s), 58° (15s) and 72° (90s). Typical secondary amplification cycling conditions were an initial denaturation at 95° for 60s, followed by 22-26 cycles of 96° (12s), 58° (15s) and 72° (40s). The resulting RT-PCR products were sequenced with dye-primer chemistry on ABI 377 sequencers. Sequences were examined for the presence of variants using the program Sequencer.

**[000193]** The primers used are set forth in Table 3. The sequence variants are set forth in Table 4.

TABLE 3

Table of Primers

<u>Name</u>	<u>Primer Sequence</u>	<u>SEQ ID NO:</u>
PDZK5.1A	CAGGTGAGGCAGGGCCGACA	10
PDZK5.1P	CTACAGTAGGCAGGGCAACAGG	11
PDZK5.1B	GTTTTCCCAGTCACGACGCGGGCTCCACCTGCTCCTC	12
PDZK5.1Q	AGGAAACAGCTATGACCATGTGAACACTAACAAACCTTTCC	13
PDZK5.1C	GTTTTCCCAGTCACGACGTCAACTCAACCATATACCCTCA	14
PDZK5.1R	AGGAAACAGCTATGACCATGGCTGGACATCCTTCACGAAG	15
PDZK5.1D	GTTTTCCCAGTCACGACGGCCTTGATTTCAGTGTGGTG	16
PDZK5.1S	AGGAAACAGCTATGACCATCCCCAACAACTGTTTCAGGC	17
PDZK5.1E	GTTTTCCCAGTCACGACGCCAGGGAACCAAGTCCACACA	18
PDZK5.1T	AGGAAACAGCTATGACCATCCTGACTGAATTCCCACAG	19
PDZK5.2A	TCCTGGAGGATTAGCAGATCGAG	20
PDZK5.2P	GGTAATCCAAAATGCTGAATCCCA	21



<u>Name</u>	<u>Primer Sequence</u>	<u>SEQ ID NO:</u>
PDZK5.2B	GTTTTCCCAGTCACGACGAAGATTGGTGGCACAAACGTG	22
PDZK5.2Q	AGGAAACAGCTATGACCATAGCACTGCCAGGTATTATACTT	23
PDZK5.2C	GTTTTCCCAGTCACGACGAGAATTGTTGGCTATGTTGGAAC	24
PDZK5.2R	AGGAAACAGCTATGACCATGCTCCAGTTAGAAAGAGAGCTG	25
PDZK5.2D	GTTTTCCCAGTCACGACGACATCCTCATCTACTTCTCCA	26
PDZK5.2S	AGGAAACAGCTATGACCATAACTCAGCATCATCTGCAATC	27
PDZK5.2E	GTTTTCCCAGTCACGACGGGAAAACCTGTTGGGTCCTG	28
PDZK5.2T	AGGAAACAGCTATGACCATCGACAGCAAACCAAAGTAAAAGG	29
PDZK5.3A	GTGGATTCTTTGATGGGCACC	30
PDZK5.3P	CTTTGAGCCACAACAGGAAGGTC	31
PDZK5.3B	GTTTTCCCAGTCACGACGTGAGCTGCTTGAGGTCAATGG	32
PDZK5.3Q	AGGAAACAGCTATGACCATCTAAAGGGTCCTGGTAATCC	33
PDZK5.3C	GTTTTCCCAGTCACGACGCCCCTGAAGTCAAGATTGTTG	34
PDZK5.3R	AGGAAACAGCTATGACCATACTTTCTTCTTCATTATCTTCC	35
PDZK5.3D	GTTTTCCCAGTCACGACGGAAATATTGAAAGCTGTGCC	36
PDZK5.3S	AGGAAACAGCTATGACCATGTCAGAAATTCATGCATCTCC	37
PDZK5.3E	GTTTTCCCAGTCACGACGAAAGTCTTTCCATTCCCAACAA	38
PDZK5.3T	AGGAAACAGCTATGACCATCCATACGGCTGTGCCTCCTG	39
PDZK5.4A	GAGTTATATCAAGATCCCTCACCAT	40
PDZK5.4P	CAAATATGCTCATGCGTGATCGG	41
PDZK5.4B	GTTTTCCCAGTCACGACGTTCACTTTGGTACACAGTGGTTG	42
PDZK5.4Q	AGGAAACAGCTATGACCATAAATCTTCTTGCTCCCTCCTT	43
PDZK5.4C	GTTTTCCCAGTCACGACGCCCGAATGATGTCCAAGGTCC	44
PDZK5.4R	AGGAAACAGCTATGACCATGTCCACCAACAATACTGATCC	45
PDZK5.4D	GTTTTCCCAGTCACGACGAGCCACTGGGGTCCACCGAG	46
PDZK5.4S	AGGAAACAGCTATGACCATACTCGTGGAGTGGATGACAAAC	47
PDZK5.4E	GTTTTCCCAGTCACGACGCAGTTGAGGCCATTAAGAAT	48
PDZK5.4T	AGGAAACAGCTATGACCATCAAGTTCAATAATGTGCAGTTCT	49
PDZK5.5A	CGCCAATGAAACTTCCTCCTCCT	50
PDZK5.5P	TCTCCTGTGAGGCATTTCTCATG	51
PDZK5.5B	GTTTTCCCAGTCACGACGCCTTTACCGACCAAAAAATCAGA	52
PDZK5.5Q	AGGAAACAGCTATGACCATCTGATTGACTGCATCCTCG	53

TABLE 3 (Con't.)  
Table of Primers

<u>Name</u>	<u>Primer Sequence</u>	<u>SEQ ID NO:</u>
PDZK5.5C	GTTTTCCCAGTCACGACGCATCTGCCATTATTAAGACTGC	54
PDZK5.5R	AGGAAACAGCTATGACCATGTGAAGTCTGCATCTGTTGAAT	55
PDZK5.5D	GTTTTCCCAGTCACGACGTCCAACAAAAGTCTCCTTCAGT	56
PDZK5.5S	AGGAAACAGCTATGACCATAACCTCTAATATCTGGTCACC	57
PDZK5.5E	GTTTTCCCAGTCACGACGCTATAGTTATCCATGAAGTCT	58
PDZK5.5T	AGGAAACAGCTATGACCATCCGCCTTTCACGATGTCAG	59
PDZK5.6A	GAAGGTGCGGCTGGTGGTGTAT	60
PDZK5.6P	CTTGCTCTGTCACCCAGGCTG	61
PDZK5.6B	GTTTTCCCAGTCACGACGGGCCTGAGCATCGTTGGGAA	62
PDZK5.6Q	AGGAAACAGCTATGACCATAACCAGGTTTTGCAGGCCAGT	63
PDZK5.6C	GTTTTCCCAGTCACGACGTCAGGGTAGTCAGCAGAGTGC	64
PDZK5.6R	AGGAAACAGCTATGACCATTACCCACATCCGCGTGAGAC	65

TABLE 4

Sequence Variants

<u>Cell line</u>	<u>Type</u>	<u>nt variant</u>	<u>aa change</u>	<u>note</u>
MDA-MB-231	breast	G1021A	gly->arg	Heterozygous variant <sup>1</sup> .
A172	glioblastoma	A1199C	glu->ala	Heterozygous variant <sup>2</sup> .
A172	glioblastoma	A1312G	ile->val	Heterozygous polymorphism
T98G	glioblastoma	A1312G	ile->val	Heterozygous polymorphism
T98G	glioblastoma	A3646G	ser->gly	Heterozygous polymorphism
NIH OVCAR-3	ovarian	A3646G	ser->gly	Heterozygous polymorphism
MDA-MB-231	breast	A3646G	ser->gly	Heterozygous polymorphism
NIH OVCAR-3	ovarian	A3959G	his->arg	Homozygous variant <sup>3</sup> .
U-373MG	glioblastoma	G4053A	none	Heterozygous polymorphism
U-118MG	glioblastoma	G4053A	none	Heterozygous polymorphism
T98G	glioblastoma	G4053A	none	Heterozygous polymorphism
MDA-MB-231	breast	G4053A	none	Homozygous polymorphism <sup>4</sup>
U-118MG	glioblastoma	C4192G	leu->val	Heterozygous polymorphism
T98G	glioblastoma	C4192G	leu->val	Heterozygous polymorphism
NIH OVCAR-3	ovarian	C4192G	leu->val	Heterozygous polymorphism
HS700T	pancreatic	C4192G	leu->val	Heterozygous polymorphism
HS700T	pancreatic	A4674G	none	Heterozygous polymorphism

<sup>1</sup> This non-conservative amino acid substitution in *MMSC1* PDZ domain #2 is at a glycine residue found at this position in most of the PDZ domains of *MMSC1*.

<sup>2</sup> This non-conservative amino acid substitution is located just before *MMSC1* PDZ domain #3

<sup>3</sup> This is a semi-conservative amino acid substitution in *MMSC1* PDZ domain #7. This particular PDZ domain has a high affinity for the C-terminus of *MMAC1*. If the amino acid substitution interferes with binding by *MMAC1*, it could be tumorigenic. That the observed variant appears homozygous raises the possibilities that the variant is either hemizygous and the cell line is LOH on the other allele at this position or that the other allele harbors a nearby splice defect. Both of these possibilities are in accord with the notion that this amino acid substitution is deleterious.

<sup>4</sup> That the observed polymorphism appears homozygous raises the possibilities that the polymorphism is either hemizygous and the cell line is LOH on the other allele at this position or that the other allele harbors a nearby splice defect. Both of these possibilities are in accord with the notion that G1021A variant in this cell line is deleterious.

## EXAMPLE 6

Generation of Polyclonal Antibody Against MMSC1

[000194] Segments of *MMSC1* coding sequence are expressed as fusion protein in *E. coli*. The overexpressed protein is purified by gel elution and used to immunize rabbits and

mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

**[000195]** Briefly, a stretch of *MMSC1* coding sequence is cloned as a fusion protein in plasmid PET5A (Novagen, Inc., Madison, WI). After induction with IPTG, the overexpression of a fusion protein with the expected molecular weight is verified by SDS/PAGE. Fusion protein is purified from the gel by electroelution. Identification of the protein as the *MMSC1* fusion product is verified by protein sequencing at the N-terminus. Next, the purified protein is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter. This procedure is repeated to generate antibodies against the mutant forms of the *MMSC1* gene product. These antibodies, in conjunction with antibodies to wild type *MMSC1*, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

#### EXAMPLE 7

##### Generation of Polyclonal Antibody

##### Against *MMSC1*-*MMSC1* Interacting Protein Complex

**[000196]** *MMSC1* is capable of binding to certain proteins, e.g., *MMAC1*. A complex of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein complex can be stabilized by cross-linking the proteins in the complex by methods known to those of skill in the art. The protein complex is used to immunize rabbits and mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

**[000197]** Briefly, the purified protein complex is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant

followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

**[000198]** This procedure is repeated to generate antibodies against forms of the complex which comprise mutant MMSC1 or mutant MMSC1 interacting protein (e.g., mutant MMAC1). These antibodies, in conjunction with antibodies to wild type MMSC1 or MMSC1 interacting protein (e.g., MMAC1), are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

## EXAMPLE 8

### Generation of Monoclonal Antibodies Specific for MMSC1

**[000199]** Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising intact MMSC1 or MMSC1 peptides (wild type or mutant) conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known.

**[000200]** The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 µg of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

**[000201]** Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of  $2 \times 10^5$  cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of MMSC1 specific antibodies by ELISA or RIA using wild type or mutant MMSC1 target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

**[000202]** Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development.

## EXAMPLE 9

### Generation of Monoclonal Antibodies Specific for MMSC1-MMSC1 Interacting Protein Complex

**[000203]** Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising MMSC1-MMSC1 interacting protein complexes (wild type or mutant), such as MMAC1, conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known. The complexes may be stabilized by cross-linking.

**[000204]** The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 µg of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

**[000205]** Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of  $2 \times 10^5$  cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of MMSC1-MMSC1 interacting protein complex specific antibodies by ELISA or RIA using wild type or mutant MMSC1-MMSC1 interacting protein complexes as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

**[000206]** Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development. Antibodies are tested for binding to MMSC1 alone

or to MMSC1 interacting protein alone to determine which are specific for the complex as opposed to binding to the individual proteins.

#### EXAMPLE 10

##### Sandwich Assay for MMSC1

[000207] Monoclonal antibody is attached to a solid surface such as a plate, tube, bead or particle. Preferably, the antibody is attached to the well surface of a 96-well ELISA plate. 100  $\mu$ L sample (e.g., serum, urine, tissue cytosol) containing the MMSC1 peptide/protein (wild-type or mutants) is added to the solid phase antibody. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100  $\mu$ L of a second monoclonal antibody (to a different determinant on the MMSC1 peptide/protein) is added to the solid phase. This antibody is labeled with a detector molecule (e.g.,  $^{125}$ I, enzyme, fluorophore, or a chromophore) and the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted and the solid phase is washed with buffer to remove unbound material.

[000208] The amount of bound label, which is proportional to the amount of MMSC1 peptide/protein present in the sample, is quantified. Separate assays are performed using monoclonal antibodies which are specific for the wild-type MMSC1 as well as monoclonal antibodies specific for each of the mutations identified in MMSC1.

#### EXAMPLE 11

##### Sandwich Assay for MMAC1 Using MMSC1

[000209] MMSC1 or PDZ domain 6 of MMSC1 is attached to a solid surface such as a plate, tube, bead or particle. Preferably, MMSC1 or its PDZ domain is attached to the well surface of a 96-well ELISA plate. 100  $\mu$ L sample (e.g., serum, urine, tissue cytosol) containing the MMAC1 peptide/protein (wild-type or mutants) is added to the solid phase MMSC1. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100  $\mu$ L of a monoclonal antibody to MMAC1 is added to the solid phase. The antibody is labeled with

a detector molecule (e.g.,  $^{125}\text{I}$ , enzyme, fluorophore, or a chromophore) and the solid phase with the antibody is incubated for two hrs at room temperature. The antibody is decanted and the solid phase is washed with buffer to remove unbound material. The amount of bound label, which is proportional to the amount of wild-type MMAC1 present in the sample, is quantified.

## EXAMPLE 12

### Drug Screening

**[000210]** The invention is useful in screening for drugs which can overcome mutations in MMSC1 and also mutations in MMAC1. The knowledge that MMSC1 and MMAC1 form a complex is useful in designing such assays. If a mutation is present in either MMSC1 or in MMAC1 which prevents the MMSC1-MMAC1 complex from forming, drugs may be screened which will overcome the mutation and allow the protein complex to form and to be active. Such screening assays can be, e.g., a yeast two hybrid assay which is dependent upon two proteins interacting. In such an assay, the presence of a mutant protein may show no activity or low activity in such an assay, while the presence of a useful drug will result in formation of a proper complex which results in activity in the assay.

**[000211]** A simple binding assay which shows the binding, i.e., formation of a complex, can similarly be used as outlined above. Useful drugs will increase the formation of MMSC1-MMAC1 complexes. Antibodies may also be used to monitor the amount of complex present. Antibodies specific for the complex are especially useful. If the presence of a drug increases the amount of complex present then the drug is a good candidate for treating the cancer which is a result of the mutation in either the MMSC1 or the MMAC1.

**[000212]** While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.



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#### Patents and Patent Applications:

European Patent Application Publication No. 0332435.

European Patent Application Publication No. 225,807.

European Patent Application Publication No. 425,731.

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